Closed Loop Control of Perfusion Systems in High-density Cell Culture

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Abstract: The use of a perfusion system prevented unspecific inhibitions effects on cell growth for batch cultivations of adherent MDCK cells used for an equine influenza A vaccine production process. It also increased viable cell numbers. An enzyme sensor for monitoring and closed loop control of metabolites operated stable for at least 55 hours.

Key words: Bioprocess, vaccine, influenza, virus, microcarrier, mammalian cell culture, Madin-Darby canine kidney, cell growth, perfusion, closed loop, open loop, dilution rate, enzyme sensor, on-line monitoring, YSI 2700, bioreactor

1. INTRODUCTION

The aim of our group is the development and optimisation of integrated concepts for vaccine production with a process of influenza A virus as an example [1]. Clearly, cell number at time of infection is one of the most important factors for achieving high virus yields [4]. Here, we present results obtained for different control strategies of perfusion systems for growth of MDCK cells.

2. MATERIAL AND METHODS

Madin-Darby canine kidney (MDCK) cells were obtained from ECACC (No. 84121903). Cultivations were performed in stirred-tank bioreactors (5 L Biostat C, B.Braun; 0.5 L Sixfors bioreactor, Infors AG) using GMEM (Invitrogen) and Cytodex 1 microcarriers (GE Healthcare) under the

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following conditions: 37°C, 50 rpm; pH = 7.3; pulsed aeration of oxygen controlled at pO_2 = 40%.

3. RESULTS AND DISCUSSION

So far, batch cultivations with higher microcarrier concentrations of up to 5 g/L did not show an expected increase in cell numbers due to media limitations but also unspecific inhibition effects [1]. To overcome these limitations the use of a perfusion system was investigated.

As a starting point, we set up an open loop perfusion system with 4 g/L MC in a 5 L STR (Table 1: run 1) and observed an increase in cell number up to 95% of the theoretical maximum cell number (3.5 x 10^6 1/mL, data not shown).

Based on these results we performed two perfusion cultivations with 5 g/L MC and a closed loop control of lactate (run 2) or glucose (run 3) in 0.5 L bioreactors to reduce media consumption.

In run 2 we had chosen a set point for lactate concentration of 15 mM to avoid growth-inhibiting concentrations reported by Hauser [3]. For on-line monitoring a sample from the outlet of the bioreactor was analysed every 30 min in an enzyme sensor. We achieved up to 84% of theoretical maximum cell number (3.94 x 10^6 1/mL), but with a high medium consumption up to 14 reactor volumes. The enzyme sensor worked stable over a period of 55 hours.

In run 3 a set point of 10 mM glucose was chosen in respect to the quantitation limit of the enzyme sensor and results from Glacken [4] regarding changes in cellular metabolism at lower glucose concentration. We achieved cell numbers up to 99% of theoretical maximum cell number (4.67 x 10^6 1/mL, Figure 1). The on-line signal of glucose showed oscillations of about ±1 mM in respect to the set point due to the dead volume of tubing (≈ 5 mL) between bioreactor and enzyme sensor.

4. CONCLUSIONS

MDCK cells showed no growth limitations for lactate concentrations up to 40 mM. We observed a stable operation of the enzyme sensor during perfusion mode for about 55 hours. The cultivations in perfusion mode achieved clearly higher cell numbers in comparison to batch cultivations [1]. Work is in progress to improve monitoring of metabolites by the use of a dead-volume free, and automated sampling device [5].

Table 1. Configuration for perfusion cultivations of MDCK cells on microcarriers in different stirred-tank bioreactors.

Run	vw (L)	D (h^{-1})	Control of ...	Controller	Feed mode
1	5	0.03	None	None	Continuous
2	0.5	0.25 (average)	Lactate	Integral	Continuous
3	0.5	0.04 (average)	Glucose	Proportional	Pulsed

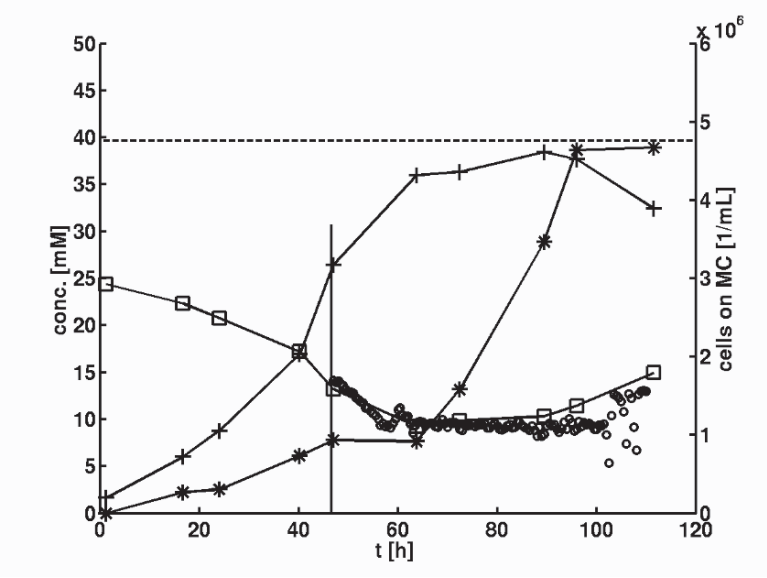

Figure 1. Cultivation of MDCK cells in perfusion mode with a closed loop control of glucose (5 g/L MC, run 3): Horizontal line: theoretical maximum cell number, vertical line (47 h): start of perfusion mode and on-line monitoring of glucose, cell number on MC (*) and metabolite profiles: glc off-line (□), lac off-line (+), glc on-line (o).

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